

## Mini-Symposia Title:

Lens-free/ computational microscopic imaging techniques for biomedical applications

## Mini-Symposia Organizer Name & Affiliation:

Kiyotaka Sasagawa, Nara Institute of Science and Technology

## Mini-Symposia Speaker Name & Affiliation 1:

Ashok Veeraraghavan, Rice University, USA

## Mini-Symposia Speaker Name & Affiliation 2:

Chanhyuk Lee, Korea Institute of Science and Technology

## Mini-Symposia Speaker Name & Affiliation 3:

Hyeon Kim, The University of Tokyo

## Mini-Symposia Speaker Name & Affiliation 4:

Benoit Gosselin, Laval University/Soo

## Mini-Symposia Speaker Name & Affiliation 5:

Kiyotaka Sasagawa, Nara Institute of Science and Technology

## Mini-Symposia Speaker Name & Affiliation 6:

## Theme:

- 01. Biomedical Signal Processing
- 02. Biomedical Imaging and Image Processing
- 03. Micro/ Nano-bioengineering; Cellular/ Tissue Engineering &
- 04. Computational Systems & Synthetic Biology; Multiscale modeling
- 05. Cardiovascular and Respiratory Systems Engineering
- 06. Neural and Rehabilitation Engineering
- 07. Biomedical Sensors and Wearable Systems
- 08. Biorobotics and Biomechanics
- 09. Therapeutic & Diagnostic Systems and Technologies
- 10. Biomedical & Health Informatics
- 11. Biomedical Engineering Education and Society
- 12. Translational Engineering for Healthcare Innovation and Commercialization

## Mini-Symposia Synopsis— Max 2000 Characters

Recent advances of image sensors and computational techniques enable high performance microscopic imaging with simple optics without any lens. By overcoming the limitation of focusing lens, compact, high resolution, wide field-of-view imaging devices can be realized. Such techniques would open a new paradigm in biomedical research. In this mini-symposium, we would like to focus on the recent activities related to the above-mentioned issues.

# **FlatCam: Beyond the limits imposed by lenses using lensless imaging systems**

Ashok Veeraraghavan and Vivek Boominathan, Rice University

The *lens* has long been a central element of cameras and microscopes, since its early use in the mid-nineteenth century by Niepce, Talbot, and Daguerre. The role of the lens, from the Daguerreotype to modern digital cameras and microscopes, is to refract light to achieve a one-to-one mapping between a point in the scene and a point on the sensor. This effect enables the sensor to compute a particular two-dimensional (2D) integral of the incident 4D light-field. However, lens-based imaging systems impose many limitations such as large size, and weight and limited field of view. We propose a radical departure from this practice and overcome the many limitations it imposes. In the talk, we focus on biomedical applications of going beyond lens-based imaging.

First, we discuss our lab's recent efforts to build flat, extremely thin imaging devices by replacing the lens in a conventional camera with an amplitude mask and computational reconstruction algorithms. These lensless cameras, called FlatCams can be less than a millimeter in thickness and enable applications where size, weight, thickness or cost are the driving factors. We demonstrate the utility of this technology for several biomedical imaging applications including lab-on-chip microscopy, in-vivo cellular-resolution microscopy, and endoscopy.

# **A Minimally Invasive, Lens-less, Single Photon Avalanche Diode CMOS Neural Imaging Probe**

Changhyuk Lee, Brain Science Institute, Korea Institute of Science and Technology, Seoul, Korea

Optical neural imaging, in-vivo calcium imaging, has revolutionized neuroscience with genetically targeted optical reporters that enable a single-cell level spatial resolution to monitor neural activity. Optics, including state-of-the-art microscopy methods, however, are fundamentally limited in imaging deep owing to a substantial scattering in tissue even with the use of the most advanced microscopy. To address the issue, here we propose a minimally invasive implantable lens-less image sensor, integrating diffractive optics, detector array, signal processing all in one CMOS IC. We discuss the perspective of implantable lens-less imaging using our prototype neural imaging probe comprises of 512 single-photon avalanche diodes distributed along two shanks. The talk covers IC design, post-processing, time-domain excitation rejection as an alternative to a spectral filter, and computational reconstructing method to demix the positions of calcium labeled neurons using sparsity regulated basis pursuit.

# Parallelized fluorescence detection using arrayed SPAD for single cell analysis

Soo Hyeon Kim

Institute of Industrial Science, The University of Tokyo  
Japan Science and Technology Agency PRESTO

High throughput discrimination and analysis of individual cells are required to detect and analyze rare cells at the single-cell level. In this talk, I will introduce advanced biomedical microsystems utilizing microfluidics and CMOS integrated circuit (IC) for single cell discrimination and analysis. For the high throughput discrimination of individual cells based on fluorescently labeled markers, parallelized flowcytometry system is developed by utilizing the CMOS IC containing an array of single photon avalanche diodes (SPADs) coupled with parallelized microfluidic channels. To realize parallelized detection of fluorescence signals without complex microscopy and optical filters, the emitted fluorescence photons are separated from excitation photons in time-domain by using fast gating and active quenching circuits in the system. The feasibility of the parallelized flowcytometry system was successfully demonstrated by detecting fluorescence signal from multiple cells labelled with fluorescent dye, which are simultaneously flowing in parallel microfluidic channels. The system will be integrated with the electroactive microwell array which allows downstream analysis of intracellular materials of single cells, and will be applied to the liquid biopsy for detecting rare cells in a body fluid.

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F. A. Author is with the National Institute of Standards and Technology,  
Boulder, CO 80305 USA, phone: 303-555-5555; e-mail: author@  
boulder.nist.gov).

# Wireless Electro-Optic Platform for Deep Brain Fiber Photometry

Benoit Gosselin, Laval University

This talk presents an optoelectronic neuroscience platform for monitoring neural dynamics in intact deep brain structures. A wireless optoelectronic headstage incorporating all aspects of a conventional benchtop high-performance optical fiber photometry system is presented to detect fluorescence signal fluctuations in the brain of live animal models. The headstage includes a photosensor, a fiber optic-cannula, an excitation light source, a few passive optical components, a microcontroller, a LED driver, and a wireless transceiver. All components are enclosed within a compact and light weight 3D-printed plastic housing. The performance of the headstage was validated in-vitro with a mouse brain slice expressing GCaMP6, a genetically encoded fluorescence indicator. The proposed headstage presents a sensitivity of 50 fA over a bandwidth of 30 Hz with an excitation light power of 50 W. The total power consumption of the headstage is approximately equal to 600 mW. The headstage prototype results in a lightweight (3.6g, w/o battery) and compact device (22 x 8 x 17 mm<sup>2</sup>), which is mountable on the head of a small laboratory animal.

# Lens-free fluorescent microscope with hybrid emission filter

Kiyotaka Sasagawa<sup>1</sup>, Makito Haruta<sup>1</sup>, Hironari Takehara<sup>1</sup>, Takashi Tokuda<sup>2</sup>, Jun Ohta<sup>1</sup>  
<sup>1</sup>Nara Institute of Science and Technology, <sup>2</sup>Tokyo Institute of Technology

**Abstract—** We recently developed a high-performance emission filter for lens-free fluorescence imaging. The filter is composed of the combination of interference and absorption filters, and the excitation rejection ratio is approximately  $10^8:1$ . We demonstrate performance improvement and fluorescence imaging of green fluorescent protein in a brain slice.

## I. INTRODUCTION

Lens-free imaging microscopy enables wide field-of-view with a relatively small device [1]. Recently, many groups have reported lens-free imaging systems. However, most reports address bright-field imaging research. This is because the emission filter performance was lower than lens-based fluorescence microscope. The interference emission filters do not work well with a lens-free setup. To solve this problem, we recently proposed a hybrid emission filter [2] and achieved excitation rejection ratio of approximately  $10^8:1$

## II. METHODS

With an interference filter, it can realize very high wavelength selectivity and extremely low transmission for the rejection band. However, the transmission spectrum shifts with the angle of the incident. Even if it reflects almost all the excitation light with normal incidence, the highly angled light can pass through the filter. This problem is solved by using a lens in a fluorescent microscope. However, in the lens-free setup, some of the excitation light is scattered by the observation target and passes through the interference filter.

To avoid this setup, we proposed to use a composite filter made of interference filter and absorption filter [3]. With this setup, the excitation light through the interference filter is eliminated by the absorption filter. Thus, high excitation rejection ratio can be achieved in the lens-free fluorescence device.

## III. RESULTS

We demonstrated fluorescence imaging by using the fabricated device. The observation target is a brain slice of a mouse developed with green fluorescent protein (GFP). Figures 1 show the imaging result.

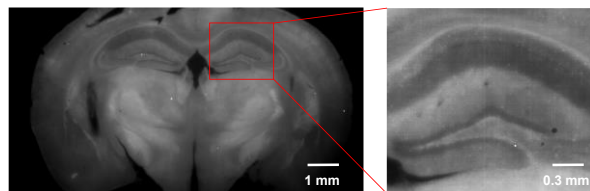


Figure 1. Fluorescence image obtained by using the fabricated lens-free fluorescent imaging device.

By using a large image sensor, wide field-of-view of  $67\text{mm}^2$  ( $=11.2\text{ mm} \times 6.0\text{ mm}$ ) has been achieved. Also, GFP developed cells were successfully observed by using the proposed device.

## IV. DISCUSSION & CONCLUSION

We developed a high-performance emission filter for a lens-free fluorescence imaging device and demonstrated the observation of fluorescent cells. With this technique, small and highly sensitive imaging devices can be fabricated. And it is expected to apply to perform long-term observation of labeled cell activities in ab incubator, *in-vivo* mouse brain fluorescence imaging and so on.

## REFERENCES

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- [2] K. Sasagawa, Y. Ohta, M. Kawahara, M. Haruta, T. Noda, T. Tokuda, J. Ohta, "Wide field-of-view lens-free fluorescence imaging device with hybrid bandpass emission filter," *AIP Advances*, vol. 9, no. 3, 035108, Mar. 2019.
- [3] K. Sasagawa, A. Kimura, M. Haruta, T. Noda, T. Tokuda, J. Ohta, "Highly sensitive lens-free fluorescence imaging device enabled by a complementary combination of interference and absorption filters," *Biomed. Opt. Express*, vol. 9, no. 9, pp. 4329–4344, Sep. 2018.

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Kiyotaka Sasagawa is with the Nara Institute of Science and Technology, Ikoma, Nara 630-0192 Japan, phone: 81-743-72-6050; e-mail: sasagawa@ms.naist.jp).